ORIGINAL ARTICLE

# Risk Factors Associated with Detection of *Salmonella* in Broiler Litter at the Time of New Flock Placement

V. V. Volkova[1], R. W. Wills[2*], S. A. Hubbard[2], D. L. Magee[2], J. A. Byrd[3] and R. H. Bailey[2*]

[1] Epidemiology Group, Centre for Infectious Diseases, University of Edinburgh, Edinburgh UK
[2] Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS, USA
[3] USDA-ARS-SPARC, College Station, TX, USA

## Impacts

- Detection of Salmonella in broiler litter at the time of flock placement was strongly associated with the replacement or top dressing of litter between flocks, as well as with the use of wood at the base of the walls on the inside of the broiler house.
- The ecology of Salmonella in broiler litter appears to be complex and additionally dependent upon vector populations, farm biosecurity protocols and probably other factors not captured in this study.
- Differences between farms rather than between production complexes within a company or between companies significantly contributed to the variability in Salmonella detection in broiler litter.

**Keywords:**
*Salmonella*; broiler; litter; food safety; epidemiology; risk factor analysis

**Correspondence:**
R. Hartford Bailey and Robert W. Wills, Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, 240 Wise Center Drive, PO Box 6100, Mississippi State, MS 39769, USA. Tel.: 662-325-7726;
Fax: 662-325-4548;
E-mail: rhbailey@cvm.msstate.edu

Received for publication May 29, 2009

doi: 10.1111/j.1863-2378.2009.01323.x

## Summary

In this study, we investigated risk factors associated with the probability to detect *Salmonella* in samples of litter collected within 2 h prior to new flock placement in 76 grow-out houses on 38 conventional broiler farms located in the US states of Mississippi, Alabama and Texas. We evaluated characteristics of location and layout of the farm; area adjacent to and surrounding the house; house construction; condition and type of equipment in the house; litter management and other production, sanitation, visitation and biosecurity practices; non-broiler animal species on the farm; and weather conditions on the 3 days leading up to flock placement. Logistic regression was used to model the relationships between probability to detect *Salmonella* in litter and potential risk factors. In the screening process, each risk factor was evaluated as a single fixed effects factor in a multilevel model that accounted for variability among the sampled farms and their production complexes and companies. Of almost 370 risk factors screened, 24 were associated with the probability to detect *Salmonella* in litter. These were characteristics of the surroundings of the house, house construction and conditions, litter management, length of downtimes between flocks in the house, biosecurity and farm location. After investigation of collinearity between these variables and building of models for important risk factor categories, the list of candidate variables for the final model was refined to eight factors. The final model demonstrated that a higher probability of detecting *Salmonella* in litter was strongly associated with the use of wood to construct the base of the walls or to cover the inside of the broiler house foundation, and with the use of fresh wood shavings to top-dress or completely replace the litter between flocks.



 

## Introduction

Over the last 50 years, the southeastern states have grown to be the leading broiler production region in the USA. Economy of scale and intensive-management production systems have allowed for this growth in the broiler industry. Among the innovations developed is the use of large-capacity grow-out houses where the birds are maintained in a managed environment conducive to maximal bird performance. One integral component of this production system is a material that can be used for floor covering (litter) in the grow-out house. Such material has to be readily available, affordable and compatible for growth and health of the birds. Given that the southeastern USA is a major producer of pine trees and pine products, the broiler industry here has widely accepted the use of pine wood chips, a byproduct, for use as broiler house floor litter.

One challenge created as a result of the use of the floor litter system is the persistence of certain pathogens within the broiler houses. A major issue of food safety concern has been sub-clinical levels of *Salmonella* in broilers. The relationship between litter *Salmonella* contamination and the persistence of *Salmonella* in broiler houses and reared flocks has been extensively investigated. The presence of *Salmonella* in a grow-out house when a new flock is placed was shown to increase the likelihood of the pathogen persisting through the production life-span of the flock (Rose et al., 1999, 2003; Cardinale et al., 2004; Volkova et al., 2009). Chemical processes occurring in broiler litter with aging and litter management were shown to affect *Salmonella* survival in this matrix (Botts et al., 1952; Snoeyenbos et al., 1967; Tucker, 1967; Olesiuk et al., 1971; Turnbull and Snoyenbos, 1973; Bhatia et al., 1979; Bhatia and McNabb, 1980; Opara et al., 1992a,b, 1994; Carr et al., 1995; Rose et al., 1999, 2003; Hayes et al., 2000; Mallinson et al., 2001). *Salmonella* was shown to survive better in fresh litter than older litter. These differences were attributed to the bactericidal characteristics of the aged litter because of decreased water activity and increased levels of ammonia (Botts et al., 1952; Tucker, 1967; Turnbull and Snoyenbos, 1973). A water activity of less than or equal to 0.85 was shown to inhibit *Salmonella* growth (Opara et al., 1992b; Carr et al., 1995; Hayes et al., 2000). It was also suggested that *Salmonella* populations in the litter may be transient in their nature and depend upon repopulation from broiler faecal material (Snoeyenbos et al., 1967).

There is limited information on how the practices of cleaning and decontaminating broiler houses between flocks impact the persistence of *Salmonella* in that environment. A 2-year study in France involving 86 grow-out broiler houses investigated risk factors associated with the persistence of *Salmonella* after decontamination between flocks and found that over one-third of the houses still harboured *Salmonella* (Rose et al., 2000). The factors associated with the *Salmonella* persistency in the final model of that study were: (i) house disinfection procedures, (ii) performance of the decontamination procedures by the farm's workers rather than a contractor, (iii) a larger percentage of the area around the house accessible by trucks, (iv) a disease requiring treatment in the previous flock in the house and (v) observation of rodents by the farmer.

Numerous sampling techniques have been used to assess *Salmonella* contamination of broiler houses. In this study, we collected pooled samples of litter as detailed in Volkova et al. (2009) and in *Sampling Technique* section below. The litter samples were obtained within 2 h prior to new flock placements in 76 grow-out houses on 38 conventional broiler farms located in the US states of Mississippi, Alabama and Texas. Analyses to identify risk factors associated with the probability of *Salmonella* detection in the litter samples were conducted. The risk factors concerned: (i) location and layout of the farm, (ii) the area adjacent to and surrounding the broiler house, (iii) house construction, (iv) condition and type of equipment present in the house, (v) litter and other production management practices, (vi) other sanitation and biosecurity practices, (vii) non-broiler animal species on the farm and (viii) weather conditions on the 3 days leading up to the flock placement.

## Materials and Methods

### Sampling design

Sample collection was carried out from 2003 to 2006. Each broiler house was sampled once, and the house was the basic unit of analysis. The study was designed to model the associations between the probability to detect *Salmonella* in litter in the house at the time of new flock placement and potential risk factors with multilevel (accounting for variability among sampled farms-complexes-companies) multiple logistic regression. Using a 'rule of thumb' of having 10 subjects, in this case houses, per explanatory variable (Petrie and Watson, 1999), 76 houses were included in the study. This sample size would allow up to seven fixed effects explanatory variables to be included in the final model. This samples size was also logistically feasible. Two broiler companies participated in the study. Thirty sampled houses were on 15 farms operating within four complexes of the first company; and the other 46 houses were on 23 farms operating within six complexes of the second company. Random selection of the farms for inclusion in the study was not deemed feasible. The farms were selected by the participating companies so that the flocks placed after

collection of the litter samples, when grown, would be processed on a Monday or Tuesday (to facilitate processing of further samples collected from the flocks for other research goals). Therefore, a selection bias might have been introduced. There was complete compliance of the growers asked to participate. The authors are not aware of any differences, which would confound the results, between the farms, which were and were not suggested for inclusion in the study. We consider that the houses sampled were generally representative of conventional grow-out broiler houses in the southeastern USA during the years of study, despite the convenience sampling. The two houses sampled on a farm were usually a house on the end of a row and the adjacent house.

### Sampling technique

Four pooled litter samples were collected from each sampled house within 2 h prior to placement of a new flock. The number of pooled litter samples was chosen based on experience and practicality. Each pooled sample consisted of eight individual litter samples collected equidistantly along one of the four lines parallel to the long side of the house and extending the complete length of the house (including brooding and non-brooding areas). The four lines were spaced equidistantly across the width of the house. The individual samples collected on each parallel line were pooled into a Whirl-Pak® Bag (NASCO, Fort Atkinson, WI, USA) and then mixed to form one pooled litter sample. Samples were transported to the laboratory on wet ice. Upon arrival at the laboratory, within 8 h of the sample collection, 25 g of each pooled litter sample were placed into a Whirl-Pak® Filter Bag (NASCO); 225 ml of buffered peptone water were added, mixed for 1 min and incubated at 42°C overnight.

### Salmonella isolation and identification

*Salmonella* isolation was performed similar to that described by Rybolt et al. (2005). In short, after overnight incubation, 1 ml from each sample was transferred to 9 ml of tetrathionate (TET), vortexed and incubated at 42°C for 48 h. After incubation, 0.1 ml of the TET was transferred to 9.9 ml of Rappaport-Vassiliadis (RV) broth (DIFCO Laboratories, Detroit, MI, USA) and incubated at 42°C overnight. After incubation, one loopful of the RV was plated onto a xylose-lysine tergitol 4 (XLT4) agar plate (Remel Inc., Lenexa, KS, USA). Following overnight incubation at 37°C, the plates were examined for *Salmonella*-like colonies. A single colony was picked from a positive XLT4 plate and *Salmonella* identity was confirmed biochemically on Triple Sugar Iron and Lysine Iron Agar slants. *Salmonella* isolation was further confirmed by a slide agglutination assay using *Salmonella* O Antiserum Poly A-I & Vi (DIFCO Laboratories) as described by the manufacturer.

### Survey tools and weather data

*Checklist completed by research team on the farms*

A checklist was developed to collect information on risk factors, which could be examined directly by the research team during sampling visits to the farms. The checklist described 117 risk factors. The following were recorded for each house sampled: (a) general characteristics of surrounding landscape within 91 m (100 yards), (b) distance to and description of the nearest (observable) forest, pine plantation, body of water and public road, (c) presence of any potential man-made rodent shelter within 91 m (100 yards), (d) presence of an outside ditch system and (e) amount of standing water around the house at the time of sampling. The type of surface of the roads connecting the houses on the farm was also recorded. Further sections of the checklist described the construction, equipment and sanitary conditions of the house at the time of sampling, and some specifications of the brooding and litter management. The house construction and equipment characteristics that were recorded included: (a) presence of work-room and its equipment, (b) presence and positioning of footbaths, (c) method of fly control, (d) condition of cool cells, (e) number of fans and other ventilation system parameters, (f) composition of footing and walls, (g) designs of the feeder and drinker lines, (h) number of walk-in doors and the type of surface outside the most frequently used door and (i) ceiling type. Brooding specifications that were recorded were the quantity, material and positioning of supplementary feed lids and the proportion of the house floor used for brooding. Sanitary conditions of the house interior and equipment at the time of sampling were evaluated visually and recorded on categorical scales. These included: (a) amount of organic contamination and dust on the footing, walls, feeders, drinkers and supplementary feed lids, (b) amount of dust on the other surfaces, (c) amount of cobwebs, (d) conditions in the work-room and (e) contamination of the footbaths. Litter management particulars that were recorded were: (a) presence of new litter or freshly top-dressed shavings, (b) degree of ammonia odour, (c) amount of visible feather and waste and (d) presence of carcasses from the previous flock on the litter at the time of sampling.

Pilot testing of the checklist was conducted by epidemiologists from the research team together with the growers at several farms sampled at the beginning of the study. Very few changes were made in the original instrument and the data collected were incorporated into the main dataset.


 

*Questionnaire completed by the growers*

A questionnaire describing 245 risk factors was completed by the owners or managers of the sampled farms. The questionnaire was designed to collect information that could not be acquired directly by the research team. Regarding the house sampled, questions were raised on: (a) sanitation of the feeder and drinker lines during the downtime (between the flocks) prior to sampling, (b) sources of drinking and fogging water, (c) litter management, including procedures performed during the downtime, (d) broiler carcass disposal procedures, (e) length of the downtime prior to sampling, (f) cumulative length of the downtimes between the last three flocks reared in the house, (g) age of the house and how many years the farm had been used to grow broilers, (h) rodent control, (i) darkling beetle, fly and other insect control and (j) sanitation of the tractors and other movable equipment used in the house. Further sections of the questionnaire contained questions on: (a) numbers of farm animals other than broilers, dogs and cats on the farm, and contact of farm personnel with the other farm animals, (b) distance from the farm to the nearest broiler hatchery, processing plant, feedmill, office of a broiler company and rendering plant, (c) number of commercial, backyard, hobby chicken and other avian flocks within ¼ mile radius from the farm and (d) numbers of visitors and biosecurity procedures for farm personnel and company and non-company visitors to the farm.

Before being delivered, two pilot tests were conducted for the questionnaire. First, a series of meetings was held with two academic poultry veterinarians who were actively involved with the broiler industry in the study region and participated in the project. Some questions were re-worded or the response categorizations were structured to standardize the responses, but some questions had to be eliminated because it was impractical to obtain a consistent characterization of the risk factor sought. At the second pilot testing, a poultry veterinarian and an epidemiologist from the research team presented the questionnaire to managerial personnel of a broiler complex in the study region. Some questions were further re-worded or edited to produce the final instrument. The Mississippi State University Institutional Review Board for the Protection of Human Subjects in Research provided approval of the project (including the checklist and questionnaire used in the present analysis) via administrative review through IRB Docket #04-005. The questionnaires were completed with the written consent of each interviewee.

*Weather data*

Broiler houses in this study were sampled at different times of the year during 2003–2006. We therefore investigated possible impacts of weather on the 3 days leading up to placement of a new flock on the detection of *Salmonella* in litter at the time of placement. Weather information was extracted from Surface Airway Observation (SAO) records of the airports nearest to the sampled farms. The nearest airport was located within 97 km from 31 of 38 farms, within 97–145 km from six farms, and >145 km away from the other farm. The SAO records were provided by Mississippi State Climatologist Dr Charles Wax, Department of Geosciences, Mississippi State University. Average environmental temperature (°C), dew point temperature (°C), relative humidity (RH percent) and amount of precipitation accumulated per day (centimeters) for the 3 days (day of placement and two previous days) were obtained. A dichotomous variable was developed to show whether or not rain occurred in at least two of the 3 days.

## Statistical procedures

Logistic regression was used to model the relationships between the probability to detect *Salmonella* in litter in the house (events/trials syntax) and risk factors examined. The aim was to evaluate the relationships between the risk factors and probability of *Salmonella* detection in litter across the poultry industry (farms, complexes, companies). There were three levels of the industry's hierarchy: the farms were nested within the production complexes, and the complexes within the companies. It was expected that there was certain variation in litter *Salmonella* contamination between the farms within a complex, complexes within a company, and between the companies. To account for resulting variability in the litter *Salmonella* contamination, the hierarchically structured random effects of the farms, complexes and companies were incorporated into the basic model structure (Condon et al., 2004). The risk factors were tested as the fixed effects factor(s) in the generalized linear mixed model containing the three random effects, further designated as the basic model. The models were fitted using the GLIMMIX procedure in SAS® 9.1 software for Windows (SAS Institute Inc., Cary, NC, 2002–2003). The basic model structure allowed testing the significance of individual factor effects beyond the inherited variation in litter *Salmonella* contamination between the units at the industry's hierarchical levels. In the screening analysis, each risk factor was evaluated in the basic model as a single fixed effects factor, and if associated with the outcome ($P \leq 0.150$) was retained for further analysis. It can be expected that fewer significant risk factors were detected as compared with the case if the screening was performed while ignoring the variability in the litter *Salmonella* contamination between the industry units.

All the risk factors retained from the screening step were investigated for pair-wise collinearity for the sampled houses. Two numerical or ordered variables were considered collinear if the statistically significant pair-wise Spearman correlation coefficient ($P \leq 0.050$) was $>|0.6|$. Two dichotomous variables were considered collinear if the simple Kappa agreement coefficient between the two having an asymptotic $P \leq 0.050$ was $>|0.6|$. The Kappa statistic was viewed as a measure of agreement between the presence and absence of the two risk factors for the sampled houses beyond that occurring by chance. Each case of collinearity detected was considered individually and the actions taken are described in *Results: Risk factor modelling* section below. Interaction among the fixed effects risk factors was tested in the basic model when deemed probable.

At first, construction of a final model was attempted by including into the basic model all the candidate risk factors (i.e. retained after the screening and collinearity investigations) at once. However, this model did not converge. Consequently, the candidate risk factors were allocated into risk factor categories and a model was built for each category. If there were too few (one or two) candidate risk factors from the category, then the category model was not considered. In building a risk factor category model, all the candidate risk factors from this category were included into the basic model at once as the fixed effects factors. After each model fit, the fixed effects variable with the highest *P*-value was removed until a model with all the fixed effects factors significant at $P \leq 0.050$ was developed.

To build the final model, all fixed effects variables from the risk factor categories' models and the remaining individual candidate risk factors were offered to the basic model at once as the fixed effects factors. After each model run, the fixed effects factor with the highest *P-value* was removed until a final model with all the fixed effects variables significant at $P \leq 0.050$ was developed. Parsimonization of this full model in terms of the fixed effects predictors was considered. A limited number of tools are available to evaluate the performance of generalized linear mixed models with different sets of predictors for a given outcome. The full model and candidate reduced models were compared using: (i) Generalized Chi-Square/df (as an approximate measure of the explained residual variation), (ii) Spearman correlation coefficient between the observed and predicted response proportions (considered as an extension of the philosophy of cross-tabulation of the predicted and observed responses for a dichotomous outcome modelled with logistic regression) and (iii) simple squared deviations statistic (sum of [(observed − expected)$^2$] as suggested by Schukken et al. (2003)).

In the adopted final model, the significance of each random effects factor was evaluated with a Wald-type test with the test statistic calculated as [(parameter estimate/parameter standard error)$^2$] and assumed to follow a Chi-square distribution with one degree of freedom under the null hypothesis.

## Results

### Salmonella in litter

Of the 76 houses sampled, 28.9% yielded at least one *Salmonella*-positive pooled litter sample. In detail, *Salmonella* was present in all four pooled litter samples from 3.9% of the houses, in three out of four in 6.5% of the houses, in two out of four in 2.5% of the houses, and only in one sample from 15.8% of the houses. Of the 38 farms studied, 21.0% had *Salmonella* present in the litter in both of the sampled houses, 15.8% had *Salmonella* present in the litter of one house but not the other, and no *Salmonella* was detected in either of the two houses on the remaining 63.2% of the farms.

### Return of checklists and questionnaires

Risk factor checklists were completed by the research team for all 76 houses sampled. Two farms (four houses) were lost from the study as a result of damage from Hurricane Katrina in August 2005; the questionnaires were not administered on these farms. The questionnaires completed by the farms' owners or managers were returned for 66 sampled houses, and not returned for the other six houses.

### Description of sampled farms, houses and litter management

Sampled farms were located within 31–34° north latitude and 87.5–96.5° west longitude in the US states of Mississippi, Alabama and Texas. The range of time the farm had been used to produce broilers ranged from zero to 45 years with an average of 13 years ($n = 66$). Every farm utilized the 'all-in all-out' management system for broilers. The number of grow-out houses on the farms ranged from 2 to 16, averaging 5. The farms obtained water from municipal supply and/or ground wells. Protocols for rodent and insect control, litter management and biosecurity practices varied. While broilers were the only domestic avian species reported on the farms, other farm animal species reported, in descending order of prevalence, were cattle, horses, goats and pen-raised alligators.

All sampled houses were constructed on dirt pad foundations placed directly on native soil, were tunnel-ventilated, and were oriented lengthwise in an east to west


 

direction. Further house construction characteristics varied between the farms and sometimes between the houses on a farm. Most of the sampled houses were either 128 or 152 m in length, with the range being 110–152 m, and the width being 12 or 13.4 m (roughly 50% of the houses in each case). The average age of sampled houses was 10 years with the range being from new to 30 years ($n = 66$). Placement of the houses relative to other buildings, bodies of water and forested land varied.

The length of downtime (when the houses were empty of birds after harvest of the previous flock) prior to sampling averaged 12 days with the range being 5–26 days ($n = 64$). The litter used in all sampled houses was pine shavings. During the downtime, in 73 of the houses, the litter was mechanically conditioned by removal of the top caked portions of the litter. After the conditioning, fresh pine wood shavings were added onto the litter in 20 of the houses. The litter was new at the time of sampling in three houses – two, on one farm, were used to grow broilers for the first time, and the litter had been totally replaced in the third house on another farm. The average age of the litter sampled was 15 months and it had been used to raise an average of five to six broiler flocks ($n = 66$). The average age of the previous litter used in the houses was 26 months by the time it was totally cleaned out and replaced with the current litter ($n = 52$). During the downtime prior to sampling, a single commercially available chemical treatment for darkling beetle control was topically applied to the litter in at least 48 houses, most commonly 4 days before the new flock placement. The litter was treated for ammonia control in at least 19 houses, usually 1 week before the placement, through the topical application of either one of two commercially available products or a household chemical. Both darkling beetle and ammonia control products were applied to the litter in at least 15 sampled houses.

## Risk factor modelling

Response distributions for several risk factors described by either the checklist or questionnaire did not allow testing the significance of associations between these variables and the detection of *Salmonella* in litter with the statistical methods applied. These factors had either too few houses within one of the response categories or the majority of the houses within a response category had all or no *Salmonella*-positive litter samples. For several other risk factors, convergence of the basic model in the screening step (evaluating the variable as a single fixed effects factor in the model) was also not reached. For all such variables, if a justifiable re-categorization of the risk factor could be carried out, which most often was dichotomization, the convergence was reached and the re-categorized variable was used in further analysis.

### *Risk factors associated with the probability to detect Salmonella in litter in the screening analysis*

Risk factors associated with the probability to detect *Salmonella* in litter in the screening step of analysis are listed in Table 1. These included some characteristics of the farm layout, surroundings of the house, house construction, litter management, length of downtimes between consecutive flocks in the house, biosecurity, and farm location. Collinearity among these risk factors was investigated. Pair-wise agreement was observed between the routine usages of footbaths when entering the broiler houses by (a) farm personnel (farm owner or employees) and company personnel, (b) farm personnel and non-company farm visitors and (c) company personnel and non-company farm visitors. The usage of footbaths was negatively collinear with both the absence of routine biosecurity requirements for the farm personnel entering the houses and the usage of disposable boots by non-company visitors (i.e. the farms tended to implement either the footbaths or the other practices). A summary variable of footbath usage by the farm workers and visitors entering the broiler houses was developed and was the only variable of this group used in further modelling.

The farm latitude and longitude were also collinear. The latitude was kept for further modelling because it showed a stronger relationship with the probability of detecting *Salmonella* in litter, as well as a smaller *P*-value in the basic model in the screening step, than the longitude. It should be acknowledged that the significance of farm latitude in this analysis should be interpreted within the longitudes of sampled farms. The distances to the nearest broiler hatchery and feedmill were collinear. The latter variable had fewer missing values and was preferred to retain for further modelling.

### *Risk factor categories' models*

Seven risk factor categories were defined. The number of candidate risk factors, those associated with the probability to detect *Salmonella* in litter in the screening analysis and retained after collinearity investigations, differed between the categories: (i) surroundings of the house at the time of sampling (five factors), (ii) house construction and conditions at the time of sampling (four factors), (iii) litter management during the downtime prior to sampling (two factors), (iv) cumulative length of downtimes between grow-outs of the last three flocks reared in the house (one factor), (v) farm biosecurity (three factors), (vi) farm location relative to other poultry facilities (one factor) and (vii) geographical coordinates (one factor). A risk factor category model was developed

**Table 1.** Risk factors associated with probability of *Salmonella* detection in broiler house litter at the time of new flock placement (after accounting for variability among the grow-out farms and broiler production complexes and companies)

| Risk factor | Response | *n* | Mean (range) or count of flocks | OR (95% CI) | *P*-value |
|---|---|---|---|---|---|
| Surroundings of the house at the time of sampling | | | | | |
| A man-made potential shelter to rodents within 91 m[b] | Yes | 70 | 40 | 3.88 (0.81, 17.70) | 0.078 |
| | No | | 30 | Reference | |
| Nearest public road[b,c] | Dirt/Gravel | 67 | 10 | 12.20 (1.20, 124) | 0.035 |
| | Asphalt | | 57 | Reference | |
| Between-houses roads on the farm are gravel versus soil/vegetation | Gravel | 74 | 55 | 0.21 (0.037, 1.20) | 0.077 |
| | Soil/Vegetation | | 19 | Reference | |
| Depth of grass litter on the sides of the house[b] | Centimeters | 61 | 14 (0–50) | 1.32 (0.90, 1.95) By 5 cm | 0.153[a] |
| Standing water around the house[b] | Extensive | 70 | 11 | 6.61 (0.95, 4.600) | 0.056 |
| | Not extensive | | 59 | Reference | |
| Ditch system around the house[b] | Yes | 72 | 62 | 0.21 (0.02, 1.84) | 0.154[a] |
| | No | | 10 | Reference | |
| House construction and conditions at the time of sampling | | | | | |
| Wood or wood-covered inside base of the walls[b,c] | Yes | 71 | 37 | 11.10 (1.59, 77.80) | 0.017 |
| | No | | 34 | Reference | |
| Dirt and organic debris on footing/cover boards[b] | Extensive/ Moderate | 71 | 31 | 0.27 (0.05, 1.38) | 0.112 |
| | Not | | 40 | Reference | |
| Time since the house was washed down[b] | Months | 54 | 16.4 (0.1–120) | 1.19 (0.96, 1.47) By 6 months | 0.1021 |
| Walk-in doors other than the main/work-room door[b] | Number | 70 | 4 (2–6) | 0.51 (0.28, 0.92) | 0.026 |
| Litter management during the downtime between flocks prior to sampling | | | | | |
| Fresh top shavings or completely new litter[c] | Yes | 76 | 23 | 4.31 (0.89, 20.90) | 0.068 |
| | No | | 53 | Reference | |
| Litter treatment against ammonia volatilization[c] | Yes | 64 | 19 | 8.02 (1.62, 39.70) | 0.012 |
| | No | | 45 | Reference | |
| Cumulative downtime between grow-outs of the last three flocks in the house | | | | | |
| Cumulative downtime between grow-outs of the last three flocks[c] | Days | 62 | 33 (18–53) | 0.54 (0.27, 1.09) By 1 week | 0.084 |
| Farm biosecurity | | | | | |
| Footbaths for farm workers entering the houses | Yes | 66 | 44 | 0.21 (0.04, 1.13) | 0.067 |
| | No | | 22 | Reference | |
| No sanitary practice for farm workers entering the houses | None | 66 | 14 | 13.70 (2.47, 76.10) | 0.004 |
| | Other | | 52 | Reference | |
| Footbaths for company personnel entering the houses | Yes | 66 | 42 | 0.26 (0.05, 1.38) | 0.109 |
| | No | | 24 | Reference | |
| Footbaths for non-company farm visitors entering the houses | Yes | 66 | 40 | 0.23 (0.04, 1.30) | 0.093 |
| | No | | 26 | Reference | |
| Summary variable of footbath usage by farm personnel, company personnel, and non-company farm visitors entering the houses[b,c] | Yes | 66 | 44 | 0.21 (0.04, 1.13) | 0.067 |
| | No | | 22 | | |
| Disposable boots for non-company farm visitors entering the houses | Yes | 66 | 24 | 7.64 (1.47, 39.70) | 0.017 |
| | No | | 42 | Reference | |
| Total times per day farm workers enter the house during brooding[b] | Times per day | 66 | 4–5 (1–11) | 0.64 (0.36, 1.16) | 0.135 |
| Pet dogs allowed on the farm[b] | Yes | 66 | 42 | 4.49 (0.56, 36.20) | 0.152[a] |
| | No | | 24 | Reference | |
| Farm location relative to other poultry facilities | | | | | |
| Distance to the nearest broiler hatchery | km | 56 | 31.23 (9.65–80.45) | 1.18 (0.96, 1.45) By 5 km | 0.122 |
| Distance to the nearest broiler feedmill[c] | km | 62 | 30 (5.63–77.23) | 1.18 (0.96, 1.44) By 5 km | 0.112 |
| Geographical coordinates | | | | | |
| Latitude[c] | Degree, North | 76 | 31.95 (31.02 –33.835) | 2.7 (0.97, 7.50) | 0.057 |
| Longitude | Degree, West | 76 | 89.67 (87.78–96.42) | 0.44 (0.15, 1.31) | 0.135 |

[a]Variable with a marginal significance ($P \leq 0.150$) in the screening step retained for further analysis.
[b]Variable was used in development of corresponding risk factor category model.
[c]Variable was used in development of final model.


 

for each of the three categories with more than two candidate variables; the candidate variables used are identified in Table 1. Each of the resulting three models contained one fixed effects risk factor. The model for the house surroundings at the time of sampling category contained the surface type of the nearest public road to the house variable. The model for the house construction and conditions at the time of sampling contained the wood/wood-covered inside base of the house's walls variable. The model for farm biosecirty practices contained the summary variable of footbath usage by the farm workers and visitors entering the houses. The associations between these factors and *Salmonella* detection in litter were similar to those observed during screening.

*Final model*

The risk factors used to build the final model (fixed effects factors from the three risk factor categories' models and the rest of the candidate variables) are identified in Table 1. The variable selection led to the full final model containing three fixed effects factors, which were the risks of (a) fresh shavings added or completely new litter placed in the house during the downtime prior to sampling, (b) the wood/wood-covered inside base of the house's walls and (c) dirt/gravel versus asphalt surface of the nearest public road to the house. The Generalized Chi-Square/df of 0.41 suggested that the full model could be over-fitted. The simple squared deviations between the observed and predicted responses statistic was 0.42, and the Spearman correlation coefficient between the two was $\rho = 0.71$ ($P < 0.001$). Non-parametric kernel density estimate of the distribution of Pearson-type residuals for this model was plotted; the center of the plot was lower than zero, but the shape was skewed to the right. None of the residuals were $>|1.5|$. Plotting the predicted responses (incorporating the random effects) against the observations suggested that the full model tended to over-estimate the predicted responses when the observed outcomes were 0.00 and under-estimate when the latter were 0.25. This might have been a drawback of the statistical procedure used, or it could have been an artefact because of the large proportion of sampled houses with no *Salmonella*-positive litter samples. The full model also produced a large confidence interval for the odds ratio for the surface type of the public road nearest to the house, though the interval was similar to the screening step.

In an attempt to obtain a more parsimonious final model and overcome the over-fitting, the full model was re-fitted omitting one of the three fixed effects risk factors at a time. When the surface type of the nearest public road was omitted, the two risk factors left remained significant ($P \leq 0.050$) (adopted final model, Table 2), as opposed to the other two reduced models. The adopted final model had a larger generalized Chi-Square/df (0.47) than the full model, suggesting that some of the over-fitting had been removed. The correlation between the predicted by the adopted final model and the observed responses ($\rho = 0.78$, $P < 0.001$) was stronger than that for the full model; however, the simple squared deviations statistics (0.52) for the adopted model was slightly larger. None of Pearson-type residuals of the adopted model were $>|1.25|$. The center of the plot of the non-parametric kernel density estimate of the residuals' distribution for the adopted model was still slightly lower than zero, but was closer to zero than that for the full model. Plotting the responses predicted by the adopted model against the observations also demonstrated an improvement over the full model, but the over-estimation of the predicted responses when the observed outcomes were 0.00 was still present.

In the adopted final model (Table 2), neither the random effects of the broiler companies nor that of the complexes appeared to contribute to the variability in *Salmonella* detection in broiler litter (both mean effects were close to zero). However, the differences among grow-out farms appeared to significantly contribute to this variability (Wald-type test *P*-value = 0.015).

## Discussion

The purpose of this study was to identify physical parameters and management practices on broiler farms that

**Table 2.** Final model of risk factors associated with probability of *Salmonella* detection in broiler house litter at the time of new flock placement (after accounting for variability among the grow-out farms, and broiler production complexes and companies[a]; $n = 71$ farms)

| Fixed effects risk factor | Response | Count of flocks | Odds Ratio (95% CI) | *P*-value |
|---|---|---|---|---|
| Fresh top shavings added or completely new litter placed during the downtime between consecutive flocks | Yes | 21 | 6.57 (1.11, 38.70) | 0.038 |
| | No | 50 | Reference | |
| Wood/wood-covered inside base of the house's walls | Yes | 37 | 11.74 (1.26, 109.60) | 0.032 |
| | No | 34 | Reference | |

[a]Neither random effects of the companies nor complexes appeared to significantly contribute to the variability in the outcome, but the random effects of grow-out farms did contribute (Wald-type test *P*-value = 0.015).

impact the extent of *Salmonella* contamination of that environment at the time of new flock placement, as evidenced by *Salmonella* recovery from the samples of litter. From almost 370 variables screened, 24 demonstrated an association with the probability to detect *Salmonella* in the litter within 2 h prior to new flock placement. Conceptually, these 24 variables can be sub-divided into seven risk factor categories: (i) surroundings of the broiler house at the time of sampling, (ii) house construction and conditions at the time of sampling, (iii) litter management during the downtime (between sequential flocks reared in the house) prior to sampling, (iv) cumulative length of downtimes between grow-outs of the last three flocks reared in the house, (v) farm biosecurity, (vi) farm location relative to other poultry facilities and (vii) farm geographical coordinates. No associations were found between *Salmonella* presence in litter and the weather conditions on the 3 days leading up to new flock placement.

The list of variables associated with the probability to detect *Salmonella* in litter in the screening analysis was refined to avoid offering collinear risk factors for final model variable selection. Building of the final model was first attempted by offering all the remaining candidate risk factors to the multilevel model at once. However, convergence of this model was not reached and several approaches to further variable selection were contemplated. Building 'intermediate' models for the most important, i.e. most represented, risk factor categories was considered to be an approach that, being technically acceptable, made the greatest use of the data, achieved the goals of the study, and eased translation of the results of analysis into practical recommendations. In particular, it allowed identification of the factor(s) most significantly associated with the outcome within each important risk factor category, therefore suggesting practices for control of *Salmonella* in the field. Then, combining such factors to build a final model provided an evaluation of the relative contribution of the risk factor categories to the outcome, therefore highlighting potentially productive research directions. It should be acknowledged that this approach may have had an unknown effect on selection of the final model. Alternatively, the reader could refer to the results of the screening analysis (Table 1), or to the risk factor categories' models outlined in *Results*.

Some of the associations detected in the screening analysis appeared contradictive to what could be expected. Detecting a spurious association is not unlikely when so many individual risk factors are considered. The risk of detecting a purely spurious association, however, was somewhat decreased by introducing the random effects structure into the risk factor model – adjusting for variations in broiler litter *Salmonella* contamination between the farms within a complex, complexes within a company, and between companies. Introduction of the risk factor categories modelling stage was likely to further decrease an opportunity for a spuriously significant variable to enter the variable selection for the final model.

Three risk factor categories contained the largest numbers of individually significant variables detected in the screening analysis: (i) surroundings of the broiler house, (ii) house construction and the conditions at the time of new flock placement and (iii) farm biosecurity. A risk factor category model was built for each.

The model for the surroundings of the broiler house category was highlighted as the most significant association between *Salmonella* in litter and the surface type of the public road nearest to the house. This variable was originally selected as an indicator of remoteness of the broiler farm. It was anticipated that houses on more remote farms as indicated by dirt or gravel roads would be less likely to harbour *Salmonella*. However, the opposite was observed. One might speculate that dust arising from unpaved roads served as a vehicle of transmission and contributed to the occurrence of *Salmonella* in the litter. Alternatively, asphalt roads can be a man-made barrier disrupting access of rodents to the farms. Overall, four characteristics of the surroundings of the broiler house, associated with the probability of finding *Salmonella* in litter in the screening analysis, may be interfering with access of rodents to the house (a man-made structure which is potential shelter to rodents within 91 m (100 yards) of the house, depth of grass litter on the sides of the house, and the surface types of the roads connecting the houses on the farm and of the nearest public road). However, none of these variables was retained in the final model adopted in this study.

The use of wood boards as a base to the walls or wood-covering of the foundation inside the house was strongly associated with higher probability of *Salmonella* detection in litter. This feature was the only risk factor that remained in the model for the house construction and conditions at the time of sampling category, and was retained in the final model adopted in this study. Several explanations of its significance could be suggested. First, houses with a wood base of the walls often did not have concrete footings, which would ease the access for rodents. However, the presence or absence of a footing was not associated with the outcome. Second, more organic debris could accumulate on the wooden surfaces in comparison with bare concrete, facilitating the survival of *Salmonella* during the downtimes between flocks. However, no association was found between the detection of *Salmonella* and a subjective score of the accumulation of dirt and debris on the walls, and an opposite association was observed with a higher accumulation of dirt and


 

debris on the footing or cover-boards themselves. Third, space behind the wood-coverings and the footing could provide shelter for the darkling beetles when the litter is treated against beetle infestation or when the litter is replaced. *Salmonella enterica* has been isolated from the darkling beetles infesting broiler houses, but data are inconclusive on the role of these insects in *Salmonella* transmission within and between reared flocks (Brown et al., 1992; Hald et al., 1998; Chriel et al., 1999; Skov et al., 1999, 2004). Fourth, the materials of the walls' base may have an impact on the moisture level in the adjacent litter. Water activity in broiler litter influences the growth of *Salmonella* in this matrix (Opara et al., 1992b; Carr et al., 1995; Hayes et al., 2000).

The use of footbaths by farm personnel, company personnel, and non-company visitors to the farm prior to entry into the broiler houses were each associated with lower probability in the screening analysis to find *Salmonella* in litter. A summary variable for the use of footbaths by all people entering the houses was the only factor retained in the model for the farm biosecurity risk factor category. This suggests that footbaths for personnel entering broiler houses are effective in preventing *Salmonella* introduction into the litter. In contrast, the use of disposable boots by non-company visitors was associated with an increased risk. A single pair of disposable boots may be used by non-company visitors to visit multiple houses on a farm leading to *Salmonella* being carried between the houses; although company personnel may follow the same practice. It is also known that non-company servicemen use plastic boots provided by their employers (for example, electricity or gas company), while broiler company personnel use plastic boots provided by the broiler companies.

The final model of this study retained two risk factors: wood base or wood-covering of the inside base of the walls of the broiler house and the placement of fresh top shavings or completely new litter during the downtime between the flocks prior to sampling. Both factors were associated with higher probability to detect *Salmonella* in litter at the time of new flock placement. As mentioned earlier, the wood at the base of the walls may provide increased access or refuge for vectors of *Salmonella* such as rodents or darkling beetles. It is also reasonable that this aspect of construction could impact the moisture content of the litter. The association of new shavings, as either the top-dress or replacement litter, with increased detection of *Salmonella* might seem counter-intuitive. However, *Salmonella* has been shown to survive better in fresh broiler litter when compared with older litter, with the bactericidal characteristics of the aged litter attributed to decreased water activity and increased levels of ammonia (Botts et al., 1952; Tucker, 1967; Turnbull and Snoyenbos, 1973). Furthermore, during sampling visits, the authors observed that fresh litter was often stored outside on the farms until placed into the broiler houses, which might contribute to increased water activity as well as increased exposure to birds, rodents, and other potential vectors of *Salmonella*.

Variability among the grow-out farms appeared to significantly contribute to the variability in extent of broiler litter contamination with *Salmonella* on the day new flocks arrived at the farms. However, differences between production complexes within a company or between companies did not appear to contribute to this variability. These findings agree with the previously reported significance of the random effects of the grow-out farms for broiler flocks *Salmonella* ser. Typhimurium status in grow-out (Chriel et al., 1999; Skov et al., 1999).

Collectively, the findings of this study suggest that the ecology of *Salmonella* in the litter in broiler houses is a complex system dependent upon construction of the house, litter management, vector populations and potentially other factors not captured in this study. The latter are likely to be among the characteristics or practices of the grow-out farms.

## Acknowledgements

This work was funded by the Epidemiological Approaches for Food Safety, USDA NRICGP 32.1, 2002-02235. We thank Dr Michael Rybolt, Dr Karen Dazo-Galarneau, Ms Terry Doler and Ms Mary Ann Ballard for laboratory and logistics support of the project. We thank Dr Charles Wax and Ashley McDonald, Department of Geosciences, Mississippi State University for providing SAO records. We thank Dr Richard Minnis, Department of Wildlife and Fisheries, Mississippi State University, for his advice on the research into population ecology of rodents. VVV is thankful to Dr Martin Miller for reading through the paper and helpful discussions. We appreciate collaboration of the participating poultry companies and thank the farmers for dedicating time to complete the questionnaires.

## References

Bhatia, T. R., and G. D. McNabb, 1980: Dissemination of *Salmonella* in broiler-chicken operations. *Avian Dis.* 24, 616–624.

Bhatia, T. R., G. D. McNabb, H. Wyman, and G. P. Nayar, 1979: *Salmonella* isolation from litter as an indicator of flock infection and carcass contamination. *Avian Dis.* 23, 838–847.

Botts, C. W., L. C. Ferguson, J. M. Birkeland, and A. R. Winter, 1952: The influence of litter on the control of

*Salmonella* infections in chicks. *Am. J. Vet. Res.* 13, 562–565.

Brown, D. J., J. E. Olsen, and M. Bisgaard, 1992: *Salmonella enterica*: infection, cross infection and persistence within the environment of a broiler parent stock unit in Denmark. *Zentralbl Bakteriol* 277, 129–138.

Cardinale, E., F. Tall, E. F. Gueye, M. Cisse, and G. Salvat, 2004: Risk factors for *Salmonella enterica* subsp. *enterica* infection in senegalese broiler-chicken flocks. *Prev. Vet. Med.* 63, 151–161.

Carr, L. E., E. T. Mallinson, C. R. Tate, R. G. Miller, E. Russek-Cohen, L. E. Stewart, O. O. Opara, and S. W. Joseph, 1995: Prevalence of *Salmonella* in broiler flocks: effect of litter water activity, house construction, and watering devices. *Avian Dis.* 39, 39–44.

Chriel, M., H. Stryhn, and G. Dauphin, 1999: Generalised linear mixed models analysis of risk factors for contamination of Danish broiler flocks with *Salmonella typhimurium*. *Prev. Vet. Med.* 40, 1–17.

Condon, J., G. Kelly, B. Bradshaw, and N. Leonard, 2004: Estimation of infection prevalence from correlated binomial samples. *Prev. Vet. Med.* 64, 1–14.

Hald, B., A. Olsen, and M. Madsen, 1998: *Typhaea stercorea* (Coleoptera: Mycetophagidae), a carrier of *Salmonella enterica* serovar infantis in a Danish broiler house. *J. Econ. Entomol.* 91, 660–664.

Hayes, J. R., L. E. Carr, E. T. Mallinson, L. W. Douglass, and S. W. Joseph, 2000: Characterization of the contribution of water activity and moisture content to the population distribution of *Salmonella* spp. in commercial poultry houses. *Poult. Sci.* 79, 1557–1561.

Mallinson, E. T., S. W. Joseph, C. L. deRezende, N. L. Tablante, and L. E. Carr, 2001: *Salmonella* control and quality assurance at the farm end of the food safety continuum. *J. Am. Vet. Med. Assoc.* 218, 1919–1922.

Olesiuk, O. M., G. H. Snoyenbos, and C. F. Smyser, 1971: Inhibitory effects of used litter on *Salmonella typhimurium* transmission in the chicken. *Avian Dis.* 15, 118–124.

Opara, O. O., L. E. Carr, E. Russek-Cohen, C. R. Tate, E. T. Mallinson, R. G. Miller, L. E. Stewart, R. W. Johnston, and S. W. Joseph, 1992a: Correlation of water activity and other environmental conditions with repeated detection of *Salmonella* contamination on poultry farms. *Avian Dis.* 36, 664–671.

Opara, O. O., E. T. Mallinson, C. R. Tate, L. E. Carr, R. G. Miller, L. Stewart, C. Kelleher, R. W. Johnston, and S. W. Joseph, 1992b: The effect of exposure, storage times, and types of holding media on the draw-swab monitoring technique for *Salmonella*. *Avian Dis.* 36, 63–68.

Opara, O. O., L. E. Carr, C. R. Tate, R. G. Miller, E. T. Mallinson, L. E. Stewart, and S. W. Joseph, 1994: Evaluation of possible alternatives to double-strength skim milk used to saturate drag swabs for *Salmonella* detection. *Avian Dis.* 38, 293–296.

Petrie, A., and P. Watson, 1999: Statistics for Veterinary and Animal Science. Blackwell Science, Oxford.

Rose, N., F. Beaudeau, P. Drouin, J. Y. Toux, V. Rose, and P. Colin, 1999: Risk factors for *Salmonella enterica* subsp. *enterica* contamination in French broiler-chicken flocks at the end of rearing period. *Prev. Vet. Med.* 39, 265–277.

Rose, N., F. Beaudeau, P. Drouin, J. Y. Toux, V. Rose, and P. Colin, 2000: Risk factors for *Salmonella* persistence after cleansing and disinfection in French broiler-chicken houses. *Prev. Vet. Med.* 44, 9–20.

Rose, N., J. P. Mariani, P. Drouin, J. Y. Toux, V. Rose, and P. Colin, 2003: A decision-support system for *Salmonella* in broiler-chicken flocks. *Prev. Vet. Med.* 59, 27–42.

Rybolt, M. L., R. W. Wills, and R. H. Bailey, 2005: Use of secondary enrichment for isolation of *Salmonella* from naturally contaminated environmental samples. *Poult. Sci.* 84, 992–997.

Schukken, Y. H., Y. T. Grohn, B. McDermott, and J. J. McDermott, 2003: Analysis of correlated discrete observations: background, examples and solutions. *Prev. Vet. Med.* 59, 223–240.

Skov, M. N., O. Angen, M. M. Chriel, J. E. Olsen, and M. Bisgaard, 1999: Risk factors associated with *Salmonella enterica* serovar *typhimurium* infection in Danish broiler flocks. *Poult. Sci.* 78, 848–854.

Skov, M. N., A. G. Spencer, B. Hald, L. Petersen, B. Nauerby, B. Carstensen, and M. Madsen, 2004: The role of litter beetles as potential reservoir for *Salmonella enterica* and thermophilic *Campylobacter* spp. between broiler flocks. *Avian Dis.* 48, 9–18.

Snoeyenbos, G. H., V. L. Carlson, B. A. McKie, and C. F. Smyser, 1967: An epidemiological study of salmonellosis in chickens. *Avian Dis.* 11, 653–667.

Tucker, J. F., 1967: Survival of *Salmonellae* in built-up litter for housing of rearing and laying fowls. *Br. Vet. J.* 123, 92–103.

Turnbull, P. C. B., and G. H. Snoyenbos, 1973: The roles of ammonia, water activity, and pH in the salmonellacidal effect of long-used poultry litter. *Poult. Sci.* 12, 72–86.

Volkova, V. V., R. H. Bailey, M. L. Rybolt, K. Dazo-Galarneau, S. A. Hubbard, D. Magee, J. A. Byrd, and R. W. Wills, 2009: Interrelationships of *Salmonella* status of flock and grow-out environment at sequential segments in broiler production and processing. *Zoonoses Public Health* doi: 10.1111/j.1863-2378.2009.01263.x. In Press.


 